pre-operation antisera. Non-phosphaturic tumours, tissue controls from skin and media controls were all negative when screened with pre-absorbed pre-operation antisera. Also, all TCM and conditioned media samples were negative when screened with post-operation antisera.

Screening of TCM proteins from patient ND, and osteosarcoma cell lines HTB96 and SaOS2 with pre-absorbed pre-operation antiserum revealed two distinct immuno-positive bands at ~ 54-57 kDa and ~ 200 kDa. Patient ND tumour sample and adjacent sub-dura tissue gave much stronger 54-57 kDa signals relative to dura brain-sample conditioned-media, and no staining for the 200 kDa band was found in the dura conditioned-media. Both HCA and LCA concanavilin-A fractions contained a very strong signal for the 200 kDa band, and a reduced but visible signal at 54-57 kDa. Cell lines SaOS2 and HTB96 were also positive for the same bands, but SaOS2 conditioned media had a reduced signal for the 200 kDa band relative to TCM and HTB96.

Skin conditioned media (patient ND and BD), and media controls were negative, as were screenings with post-operation antisera (Rowe, Bone 18 (1996), 159-169). Recombinant MEPE (rec-MEPE), stained positively with pre-absorbed pre-operation antisera, and this could be competed out with added rec-MEPE). A positive band of 54-57 kDa was obtained with Sybr-Orange protein stained, and pre-absorbed pre-operation antisera screened rec-MEPE. This was the same size as the 55-57 kDa band (pre-absorbed-pre-operation western screened), found with patient ND tumour conditioned media, and osteosarcoma cell lines HTB96 and SaOS2. Recombinant-MEPE contains an additional 4.5 kDa CBP-tag at the N-terminus that decreases mobility and results in an apparent increase in molecular weight on SDS-PAGE gels. Thus, the equivalent size of tumour derived protein and rec-MEPE may be due to post-translational modification of tumour derived MEPE (possibly glycosylation).

TCM western blots from OHO-tumour patients BD and EM contained major pre-absorbed-pre-operation antisera positive bands at slightly lower molecular weight (48-52 kDa), as well as a band co-migrating at 55-57 kDa with rec-MEPE. Other

higher molecular weight bands were also seen at 61, 75, 80, and 93 kDa (weaker signals).

In all samples the major SYBR-Orange stained protein band at 66 kDa was negative when screened with pre-absorbed pre-operation antisera. Glycoprotein screening of duplicate blots gave the same results as screening with pre-operation antisera and both 54-57 kDa and 200 kDa bands stained positive confirming that these proteins are glycosylated. Proteins were separated by SDS-PAGE and blotted onto PVDF membranes as described in methods above. Specific glycoprotein detection was carried out using an Immuno-Blot kit for glycoprotein detection (Bio-Rad), and Amersham biotinylated markers were added as internal controls. Briefly, after transfer membranes were treated with 10 mM sodium periodate in sodium acetate/EDTA buffer to oxidise carbohydrate moieties. The blots were then washed in PBS and incubated with hydrazide in sodium/acetate/EDTA buffer for 60 minutes at room temperature. Filters were then washed three times (10 minutes) with TBS. Subsequent blocking and detection was carried out as described earlier using the Enhanced chemiluminescence kit (Amersham), and streptavidin horse radish peroxidase. Primary antibody and secondary goat anti-rabbit-HRP was not used.

In conclusion pre-absorbed pre-operation antisera specifically detects proteins derived from oncogenic hypophosphatemic osteomalacia-TCM. The major proteins detected fall into two three distinct molecular size ranges 48-52 kDa, 54-57 kDa, and 200 kDa. All OHO-TCM samples were positive for the 54-57 kDa protein, and all proteins detected by pre-absorbed-pre-operation antisera stained positive when screened for glycoprotein status. Non OHO-tumours control tissues and media were negative when screened with pre-absorbed pre-operation antisera.

Example 9: Expression of MEPE fusion-protein from pCAL-n-EK vector

The entire cDNA coding sequence was subcloned into pCAL-n-EK as described in Example 4a. Validation of the fusion construct generated by IPTG induction of the E. coli host BL21 (DE3), was achieved by screening western blots with pre-

operation antisera, and also with calmodulin conjugated to alkaline phosphatase as described above. The fusion protein with microbial CBP-tag (calmodulin binding peptide of 4.5 kDa), containing calmodulin peptide, enterokinase site, and thrombin site was 56 kDa as deduced by SDS-PAGE. This is in approximate agreement with the expected molecular size (~48 kDa). Purification of protein was achieved by calmodulin affinity chromatography as described above. Preincubation of pre-operation antisera with purified fusion construct resulted in a diminution of the 55-57 kDa signal observed on screening TCM western blots, but not the 200 kDa band. The failure to completely reduce the 55-57 kDa signal was presumed to be due to specific recognition of the highly antigenic glycosylation moiety present in the nascent MEPE-protein (TCM), but absent in the microbial fusion-construct of rec-MEPE. The fusion protein was soluble in aqueous Tris-buffers and detergents were not required at any stage of the purification process.

Example 10: Tissue expression (RT/PCR and Northern analysis)

Northern blots containing poly A+ RNA were screened with MEPE cDNA and no hybridization was detected to stomach, thyroid, spinal cord, lymph node, trachea, adrenal gland, bone marrow, heart, brain, lung, liver, skeletal muscle, kidney, and pancreas (Clontech MTN-blots I and III). For Northern analysis two blots from Clontech (MTN^{TM} and MTN^{TM}III), containing the following poly A^{+} RNA's: 1; heart, 2; brain, 3; placenta, 4; lung, 5; liver, 6; skeletal muscle, 7; kidney, 8; pancreas, 9; stomach, 10; thyroid, 11; spinal cord, 12; lymph node, 13; trachea, 14; adrenal gland, 15; bone marrow, were screened with MEPE cDNA amplified with specific internal primers (Pho433-111F and PHO877-111R). Primer sequences for Pho433-111F and PHO877-111R are highlighted in figure 8 (nucleotide positions 433 to 456 (SEQ ID NO: 24) and 877 to 900 (SEQ ID NO: 25), respectively), and the following PCR conditions were used: predenaturation; 95°C 3 min; followed by thirty cycles of denaturation; 95°C 45 sec, annealing; 65°C 30 sec, polymerization; 72°C 45 sec, and a final extension of 72°C 7 min followed by cooling to 4°C. PCR-buffer (PB), was used with a final concentration of 2 mM $MgCl_2$. The 444 bp amplified MEPE cDNA product was then resolved by submarine agarose electrophoresis, visualized by ethidium bromide staining, and purified using glass beads (Gene-clean II kit; Bio 101 INC). Purified DNA was then radiolabeled using